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Pharmacology

Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation

(rat/Chinese hamster ovary cells/*in situ* hybridization)

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ABSTRACT **By using a strategy based on nucleotide sequence homology, we have cloned a cDNA encoding a functional serotonin (5-HT) receptor. The deduced amino acid sequence of the 5-HT_7 receptor displays limited homology with that of other 5-HT receptors. In addition to the seven stretches of hydrophobic amino acids that characterize the superfamily of receptors interacting with guanine nucleotide-binding proteins, the 448-aa sequence of the 5-HT_7 receptor contains a hydrophobic domain located at its N-terminal end. Genomic analysis indicated the presence of introns interrupting the coding sequence. The 5-HT_7 receptor, stably expressed in transfected CHO cells, bound [^{3}H]5-HT with high affinity (K_d = 1 nM), like receptors of the 5-HT_1 subfamily from which, however, it was clearly distinguished by its pharmacology. 5-HT in nanomolar concentrations stimulated cAMP accumulation in these CHO cells by ≈10-fold, whereas lysergic acid diethylamide displayed low intrinsic agonist activity. These various properties differentiate the 5-HT_7 receptor from the four other subfamilies of mammalian 5-HT receptors (i.e., the 5-HT_1-, 5-HT_2-, 5-HT_3-, and 5-HT_4-like subfamilies) and, therefore, appear to define another receptor subfamily. Northern blot and *in situ* hybridization analyses showed the 5-HT_7 transcripts to be expressed in discrete areas of the limbic brain (e.g., pyramidal hippocampus cells, tenia tecta, amygdaloid, or mammillary nuclei), suggesting that the receptor mediates serotoninergic controls in functions like mood, learning, or neuroendocrine and vegetative behaviors.**

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that exerts its effects mainly in the central nervous and gastrointestinal systems by interacting with a large variety of receptors. These were initially distinguished through the use of traditional pharmacological approaches with isolated organs (1) and, then, in binding studies (2). More recently, with the introduction of molecular biology approaches in the field (3), a wealth of additional 5-HT receptor subtypes could be characterized.

Now, it appears that, whereas the 5-HT_3 receptor is a ligand-gated ion channel (4), mammalian 5-HT receptors with seven putative transmembrane domains (TMs) and coupled to guanine nucleotide-binding (G) proteins can be divided into three subfamilies, depending on molecular, ligand binding, and effector-coupling properties. Members of the 5-HT_1 subfamily [except, perhaps, the preliminarily characterized 5-HT_5 receptor (5)]—i.e., 5-HT_{1A} (3, 6), 5-HT_{1B} (7–9), 5-HT_{1D} (10–12), 5-HT_{1E} (13), and 5-HT_{1F} (14) receptors—are encoded by intronless genes, display nanomolar affinity for 5-HT, and are negatively coupled to adenylyl cyclase. The 5-HT_2 subfamily contains the homologous 5-HT_{1C} (15) and 5-HT_2 (16) receptors, which are characterized by a coding sequence interrupted by introns (17, 18) and a positive coupling with phospholipase C. Finally, the not yet cloned 5-HT_4 receptor (19) and the recently cloned 5-HT_6 receptor (20, 21) display submicromolar or micromolar affinity for 5-HT and are positively coupled to adenylyl cyclase.

There is evidence, however, for the existence of additional 5-HT receptors. For instance, high-affinity [^{3}H]5-HT binding sites, pharmacologically distinct from members of the 5-HT_1 subfamily (22, 23), and high-affinity 5-HT receptors positively coupled to adenylyl cyclase, presumably distinct from the 5-HT_4 receptor (24–26), have been described.

We report here the characterization of an additional 5-HT receptor belonging to the superfamily of G-protein-coupled receptors that we propose to name 5-HT_7 in accordance with recently proposed nomenclature rules (27).§ From its molecular, pharmacological, and signaling properties, it appears that the 5-HT_7 receptor may define another subfamily of mammalian 5-HT receptors.

MATERIALS AND METHODS

Cloning and Sequencing of a Rat cDNA. A rat brain cDNA library (28) was screened at low stringency with a ^{32}P-labeled DNA fragment corresponding to the coding region of the rat substance P receptor gene [nt −27 to +1275 (29)]. One clone (λSP45) was shown to hybridize weakly with an *Afl* II–*Bgl* II restriction fragment (nt 1193–1446) of the D_2 receptor gene (30). A ^{32}P-labeled DNA fragment (523 bp) of this clone was used to screen, under high stringency, a rat hypothalamus cDNA library constructed in λZAPII (Stratagene). A 1.5-kb *Hin*dIII–*Hin*dIII DNA fragment of one clone (λHPT3) among six positive identical clones, purified >2 × 10^6 times, was subcloned in pGEM-4Z (Promega) and sequenced. It encodes an open reading frame of 359 aa starting from aa 89 (Fig. 1). A ^{32}P-labeled DNA fragment encoding aa 90–193 was used to screen a partial *Hae* I-cut rat genomic library constructed in EMBL3 vector (Stratagene). Three positive clones, λHae I-a, -b, and -c, were purified >6 × 10^5 times. A 6.5-kb fragment from λHae I-a was subcloned into pGEM-4Z for sequencing. It contained an open reading frame of 182 aa starting from Met[1] in Fig. 1 and a consensus sequence for an intron in the 3′ end. An identical 1.6-kb *Hin*dIII–*Hin*dIII fragment from λHae I-b and -c was also subcloned, sequenced, and found to contain an open reading frame coding for aa 184–435 (Fig. 1). Consensus splice sites for intronic sequences were found in 5′ and 3′ ends of the coding sequence (data not shown).

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Abbreviations: 5-HT, serotonin; G protein, guanine nucleotide-binding protein; LSD, lysergic acid diethylamide; TM, transmembrane domain.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L19654).

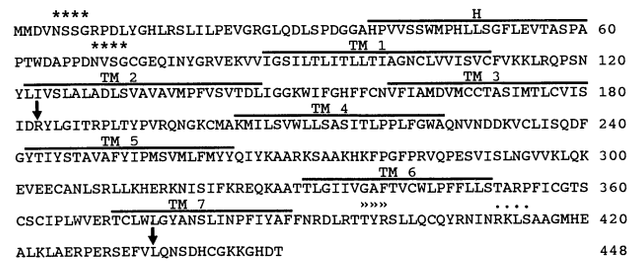

FIG. 1. Deduced amino acid sequence of the 5-HT_7 receptor. The seven TMs (TM1–TM7) characteristic of G-protein-coupled receptors and an additional hydrophobic domain (H) are overlined. Arrows indicate the positions of introns. Symbols *, ·, and ≫ represent consensus sites for glycosylation and protein kinase A and C phosphorylation, respectively.

The full-length cDNA was obtained by PCR (31). A primer, containing a *Bgl* II restriction site and located 82 nt downstream from the first in-frame TGA stop codon (primer 1), was synthesized. It was used at a concentration of 375 nM for the synthesis of a single-strand cDNA with avian myeloblastosis virus reverse transcriptase (20 units; Boehringer Mannheim) and 2 μg of rat hypothalamus poly(A)$^+$ mRNAs as template. This template was amplified using 75 nM of primer 1 and primer 2 (containing a *Hin*dIII restriction site located 44 nt, upstream of the first in-frame ATG) for 35 identical cycles (94.5°C, 56°C, and 72°C for 1.5, 1.5, and 3 min, respectively) with 5 units of *Taq* DNA polymerase (Perkin–Elmer/Cetus). PCR products were electrophoresed and a band of the predicted size (≈1500 bp) was excised, purified using Geneclean II (Bio 101), digested with *Hin*dIII and *Bgl* II, ligated into pGEM-4Z, and sequenced. The nucleotide sequence was identical to that found in λHPT3 and λHae.

Expression in CHO Cells. The expression vector pSV5-HT_7, derived from the pSVD_2 (28), was prepared (32) and constructed using the *Hin*dIII–*Bgl* II fragment described above containing the full-length coding sequence of the 5-HT_7 receptor gene. CHO-K1 cells deficient in dihydrofolate reductase were transfected (32) and stable transfectants were selected and tested for [^{3}H]5-HT binding. One clone, named CHO(5-HT_7), expressing ≈500 fmol of sites per mg of protein, was selected for further characterization.

[^{3}H]5-HT Binding Assay. Cell membranes (20–40 μg of protein) were incubated at 25°C for 30 min in 0.5 ml of 50 mM Tris·HCl (pH 7.6) containing 10 μM pargyline, 4 mM Ca^{2+}, and 0.05% ascorbate with [^{3}H]5-HT alone (total binding) or in the presence of 10 μM 5-HT (nonspecific binding). Incubations were terminated by rapid filtration (32).

cAMP Accumulation. Cells (96-well plates) were washed twice (10 min at 37°C) and incubated for 10 min at 37°C with the appropriate drugs in Dulbecco's modified Eagle's medium containing 0.1 mM isobutylmethylxanthine and 0.05% ascorbate. cAMP was extracted and measured by radioimmunoassay (DuPont; Rianen cAMP [^{125}I]radioimmunoassay kit).

Adenylyl Cyclase Activity. Membranes were prepared and incubations performed essentially as described (33). cAMP was measured by radioimmunoassay.

Northern Blot Analysis. Poly(A)$^+$ mRNAs from Wistar rats or Hartley guinea pigs were subjected to Northern blot analysis as described (32), hybridization being performed with a probe ^{32}P-labeled by nick-translation and corresponding to the nucleotide sequence encoding aa 90–448 (Fig. 1). Blots were washed three times in 2× standard saline citrate (SSC)/0.1% SDS at 42°C for 20 min and once in 0.2× SSC/0.1% SDS at 42°C for 15 min.

***In Situ* Hybridization.** Brain sections were prepared and incubated essentially as described (34). Hybridization was performed in the presence of 50% (vol/vol) formamide/10 mM dithiothreitol with a probe corresponding to aa 390–448, synthesized by PCR, and subcloned in pGEM-4Z. ^{35}S-labeled antisense- or sense-strand RNA probes were obtained using a Riboprobe kit (Promega).

RESULTS

Deduced Amino Acid Sequence of the 5-HT_7 Receptor. Sequencing of overlapping rat hypothalamus cDNA and genomic DNA fragments led to the characterization of a nucleotide sequence containing an open reading frame encoding a protein of 448 aa (Fig. 1) with an estimated molecular weight of 49,836. The coding region of the gene is interrupted by at least two introns. Analysis of λHae I-a DNA insert revealed an open reading frame of 182 aa beginning with a consensus initiator methionine (GGCACGATGATG) (35), a nonsense codon at position −75, and an intron sequence starting after aa 182 (data not shown). Sequencing the λHPT3 cDNA insert revealed an open reading frame corresponding to 359 residues (aa 89–448 in Fig. 1) and possessing a stop codon TGA. The full-length nucleotide sequence was obtained by reverse transcription of poly(A)$^+$ mRNAs from rat hypothalamus followed by PCR amplification using oligonucleotides flanking the 5′ and 3′ coding region found in λHae I-a and λHPT3 DNA fragments. Clone λSP45 was found to correspond to the C-terminal amino acid sequence starting at residue 306.

Hydropathicity analysis (36) revealed eight clusters of 20–25 hydrophobic amino acids that could span the cell membrane (Fig. 1, TM1–TM7 and H). Comparison of the amino acid sequence of 5-HT_7 receptor with G-protein-coupled receptors indicates significant homology, particularly with other 5-HT receptors. The highest overall homology (38%) was found with a 5-HT receptor of *Drosophila*, 5-HT_{dro1} (37), and homology was 60% when only TMs are considered. In these TMs, the homologies with other 5-HT receptors were as follows: 51% with 5-HT_{1A} (6), 55% with 5-HT_{1B} (9), 42% with 5-HT_{1C} (15), 52% with 5-HT_{1D} (11), 53% with 5-HT_{1E} (13), 52% with 5-HT_{1F} (14), 43% with 5-HT_2 (16), 40% with 5-HT_{2F} (38), 48% with 5-HT_5 (5), and 45% with 5-HT_6 (20, 21). Clustering of 5-HT receptors according to overall homology (39) is shown in Fig. 2. The homology was also significant with the TMs of some other receptors such as the β_2-adrenergic receptor (50%) (40), whereas it was rather low (<35%) with those of the rat substance P receptor (29).

In analogy with other members of the superfamily (41), the 5-HT_7 sequence is namely characterized by the presence of (*i*) consensus glycosylation sites in the N terminus, (*ii*) an aspartate residue (Asp^{165}) in TM3 found in all aminergic

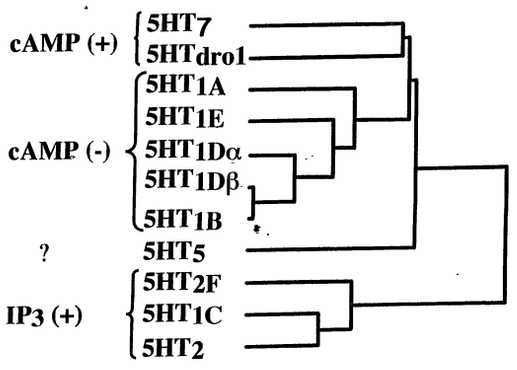

FIG. 2. Clustering of 5-HT receptor subtypes according to sequence homology and intracellular signaling system. Amino acid sequences of the rat 5-HT_7 (Fig. 1), 5-HT_{1A} (6), 5-HT_{2F} (38), 5-HT_{1C} (15), 5-HT_2 (16), human 5-HT_{1E} (13), 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (12), mouse 5-HT_{1B} (9) and 5-HT_5 (5), and *Drosophila* 5-HT_{dro1} (37) receptors were compared and clustered (39). The length of horizontal lines is inversely proportional to percent homology. cAMP (+ or −) and IP_3 (+) denote positive (+) or negative (−) coupling to adenylyl cyclase or positive coupling to phospholipase C, respectively.

receptors and thought to salt-link an ammonium group of ligands, (*iii*) two cysteine residues (Cys^{158} and Cys^{234}) presumably linked by a disulfide bond, (*iv*) two residues in TM5 (Ser^{246} and Ala^{250}) presumably involved in the binding of the indole moiety of 5-HT (42, 43), and (*v*) consensus sites for phosphorylation by protein kinases A and C in the C terminus (Fig. 1). A characteristic feature of the structure is the relatively short third cytoplasmic loop and long C-terminal tail, found in many receptors positively coupled to adenylyl cyclase (44).

Stable Expression and Properties of the 5-HT_7 Receptor in Transfected CHO Cells. CHO cells expressing the 5-HT_7 receptor were selected using a [^{3}H]5-HT binding assay. Whereas wild-type CHO cells did not express any specific [^{3}H]5-HT binding, CHO(5-HT_7) cell membranes bound [^{3}H]5-HT in a monophasically saturable manner (n_H = 0.95). Scatchard analysis of data from three experiments with 6–14 [^{3}H]5-HT concentrations (20 pM to 100 nM) and triplicate samples led to a K_d value of 1.0 ± 0.2 nM and a B_{max} value of 537 ± 66 fmol/mg of protein (data not shown). [^{3}H]5-HT binding was inhibited by a series of agents with K_i values (45) defining a pharmacological profile distinct from that of other 5-HT receptors (Table 1). The pseudo Hill coefficient of these agents did not significantly differ from unity. In few preliminary experiments using [^{3}H]spiperone as a ligand, 5-HT displaced the binding in a biphasic manner (pseudo Hill coefficient = 0.67) in the absence of guanine nucleotide; in the presence of 0.1 mM guanosine 5′-[β,γ-imido]triphosphate, the displacement curve became monophasic and the IC_{50} value was increased 2-fold (data not shown).

Table 1. Apparent dissociation constants of drugs for [^{3}H]5-HT binding sites in membranes of CHO(5-HT_7) cells

Drug	K_i, nM
5-HT	0.60 ± 0.02
5-CT	0.12 ± 0.01
5-Methoxytryptamine	0.94 ± 0.10
(±)-8-OH-DPAT	52 ± 6
(+)-LSD	9.5 ± 1.0
Lisuride	6.3 ± 0.7
Buspirone	381 ± 37
Sumatriptan	506 ± 58
Dihydroergotamine	150 ± 15
Dihydroergocryptine	310 ± 25
Metergoline	63 ± 4
Cyproheptadine	77 ± 6
Spiperone	20 ± 2
Mianserin	67 ± 7
Clomipramine	127 ± 20
Clozapine	61 ± 8
Chlorpromazine	70 ± 6
(+)-Butaclamol	90 ± 19
Haloperidol	500 ± 102
(+)-7-OH-DPAT	1,014 ± 57
(±)-Cyanopindolol	>10,000
(±)-Pindolol	>2,500
Ketanserine	>7,500
ICS 205930	>30,000
Metoclopramide	>30,000
(−)-Sulpiride	>3,000
Histamine	>10,000
Dopamine	>12,500

[^{3}H]5-HT (2 nM) was incubated in the presence of the drugs in at least six concentrations. K_i values were derived from IC_{50} values, taking into account a K_d value of 1 nM for [^{3}H]5-HT. Values (mean ± SEM) were derived from data obtained in two to four experiments with triplicate determinations. 5-CT, 5-carboxamidotryptamine; DPAT, *N,N*-di-*n*-propyl-2-aminotetraline.

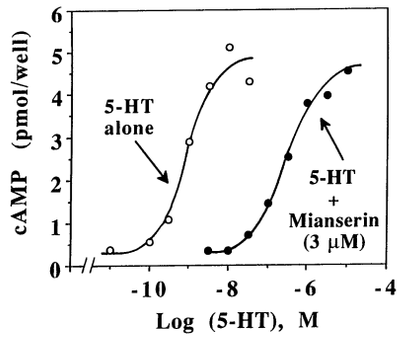

FIG. 3. 5-HT-induced stimulation of cAMP accumulation in CHO(5-HT_7) cells and its inhibition by mianserin. Data shown are the mean ± SEM of quadruplicates in a single experiment, which was repeated three times. The EC_{50} values of 5-HT were 1.2 ± 0.3 nM and 0.29 ± 0.04 μM in the absence and presence of 3 μM mianserin, respectively, leading (45) to an apparent K_i value of 14 ± 2 nM for the antagonist.

5-HT induced an ≈10-fold maximal stimulation of cAMP accumulation in CHO(5-HT_7) cells with an EC_{50} value of 1.2 ± 0.3 nM and a rightward shift of the concentration–response curve to 5-HT was observed in the presence of 3 μM mianserin (Fig. 3). In comparison, the maximal lysergic acid diethylamide (LSD)-induced stimulation was only of 232 ± 18% (n = 4) and was progressively inhibited by mianserin (Fig. 4). Stimulation by other 5-HT agonists is shown in Fig. 5. 5-HT augmented forskolin-induced accumulation of cAMP in CHO(5-HT_7) cells; 5-HT (10 nM) also stimulated adenylyl cyclase activity on membranes of these cells 1.5- to 2-fold over basal level whereas there was no stimulation on membranes of wild-type CHO cells (data not shown).

Localization of 5-HT_7 Receptor mRNAs in Rat and Guinea Pig Tissues. Northern blot analysis of a variety of rat tissues revealed two mRNAs of ≈3.9 and ≈3.1 kb (Fig. 6). The strongest signal was observed in hypothalamus, brainstem, and hippocampus, and the lowest was in stomach and ileum. Similar results were obtained with another probe corresponding to the N-terminal sequence encoding aa 1–71 in Fig. 1 (data not shown). With guinea pig tissues two bands of ≈3.8 and 3.0 kb were revealed. Highest expression was observed in thalamus, brainstem, hypothalamus, substantia nigra, olfactory bulb, and tubercle, whereas the signal was hardly detectable in peripheral organs.

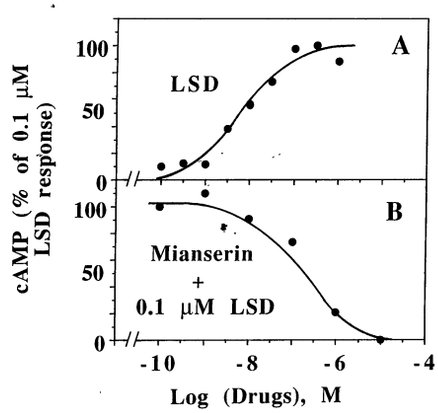

FIG. 4. LSD-induced stimulation of cAMP accumulation in CHO(5-HT_7) cells (*A*) and its inhibition by mianserin (*B*). cAMP levels in the absence and presence of 0.1 μM LSD were 0.24 ± 0.03 and 0.56 ± 0.04 pmol per well, respectively. The EC_{50} value of LSD was 8 ± 2 nM. The IC_{50} value of mianserin in presence of 0.1 μM LSD was 260 ± 80 nM, leading (45) to an apparent K_i value of 38 ± 13 nM. Data are the mean ± SEM from three or four experiments.

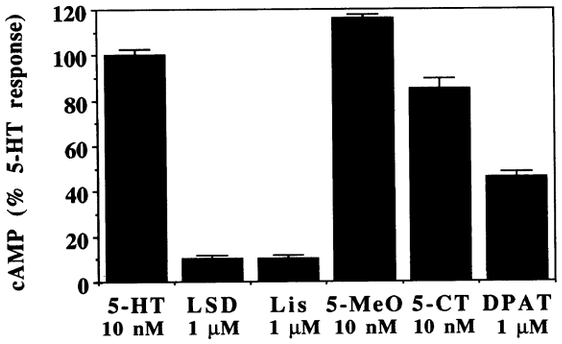

FIG. 5. Stimulation of cAMP accumulation in CHO(5-HT$_7$) cells induced by 5-HT and various agonists. The compounds were tested at concentrations corresponding to 10–100 times their K_i values in binding experiments. Data are expressed as percentages of the stimulation induced by 10 nM 5-HT in the same experiments. Lis, lisuride; DPAT, 8-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetraline; 5-MeO, 5-methoxytryptamine; 5-CT, 5-carboxamidotryptamine.

In situ hybridization with the antisense probe revealed a highly heterogeneous distribution of transcripts in rat brain. Highest levels were found in the retrosplenial cortex, hippocampus, tenia tecta, indusium griseum, and posterior hypothalamus (Fig. 7), as well as in the amygdaloid complex (medial amygdaloid nucleus), thalamus (paraventricular nucleus), cerebellum (Purkinje cell layer), and pontine nuclei (data not shown); clear hybridization signals also occurred in superior colliculus and dorsal and paramedian raphe nuclei. The use of the corresponding sense probe led to weak uniform signals (Fig. 7).

DISCUSSION

The present characterization of the 5-HT$_7$ receptor is based upon genomic, molecular, pharmacological, and signaling properties. These properties define not only another member among the already plethoric family of serotoninergic receptors coupled to G proteins but also, apparently, another subfamily.

In support to this proposal, five major properties seem to define the 5-HT$_7$ receptor and differentiate it from other 5-HT

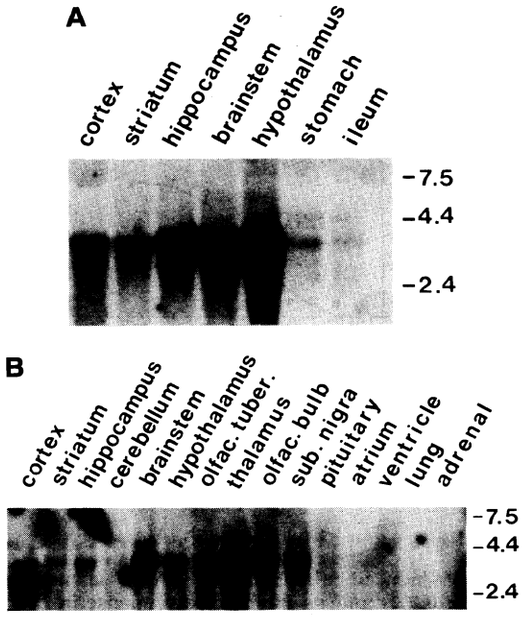

FIG. 6. Northern blot analysis of 5-HT$_7$ receptor gene transcripts in various rat (*A*) and guinea pig (*B*) tissues. Poly(A)$^+$ mRNAs (8 μg per lane) were used. Blots were exposed to x-ray films for 7 days at −80°C. Molecular sizes (kb) are shown.

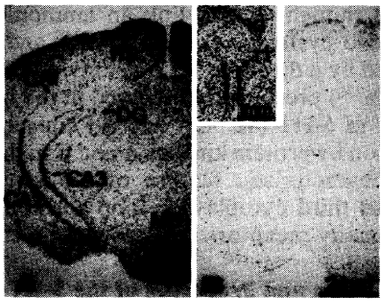

FIG. 7. Localization of 5-HT$_7$ receptor gene transcripts in frontal sections of rat brain by *in situ* hybridization. ^{35}S-labeled antisense- (*A* and *B Inset*) or sense- (*B*) strand RNA probes corresponding to the C-terminal amino acid sequence were used. CA1-3, fields CA1-3 of Ammon's horn; DG, dentate gyrus; IG, indusium griseum; MB, mammillary bodies; RS, retrosplenial cortex; TT, tenia tecta.

receptors—i.e., limited sequence homology, presence, and location of at least two introns, existence of an eighth hydrophobic domain, high-affinity binding of 5-HT, and positive coupling to adenylyl cyclase.

The highest amino acid sequence homology, found with the 5-HT$_{dro1}$ receptor (37), is only of 38%, a value that might be of significance, however, considering the phylogenic distance between *Drosophila* and rat. Since the 5-HT$_7$ and 5-HT$_{dro1}$ receptors also have in common the occurrence of an eighth hydrophobic sequence of 20–25 aa at the N-terminal part as well as the ability to stimulate adenylyl cyclase, they may represent species variants of the same receptor. Unlike the 5-HT$_7$, however, the 5-HT$_{dro1}$ receptor is activated at rather high 5-HT concentrations and is encoded by an intronless gene. The functional significance of the N-terminal hydrophobic domain, which is also found in the 5-HT$_{1c}$ receptor (17), in addition to the paradigmatic seven putative α-helices, is not immediately apparent; although its length is compatible with a membrane-spanning location, it could also function as a cleavable signal sequence (37).

Receptors of the 5-HT$_2$ subfamily (i.e., 5-HT$_{1c}$ and 5-HT$_2$ itself) also have the coding sequence of their genes interrupted by introns but the location of the latters differs (18). Their sequence homology with 5-HT$_7$ is rather low, even in rodents, and they are coupled with phospholipase C instead of adenylyl cyclase (15, 16).

Receptors of the 5-HT$_1$ subfamily (i.e., 5-HT$_{1A}$ (6), 5-HT$_{1B}$ (9), 5-HT$_{1D}$ (11), 5-HT$_{1E}$ (13), and 5-HT$_{1F}$ (14) receptors] are also characterized by their high-affinity binding of [^{3}H]5-HT, by virtue of which this subfamily was initially defined (2), but again, they differ from the 5-HT$_7$ receptor by their intronless gene, low sequence homology, and negative coupling to adenylyl cyclase (Fig. 2).

The 5-HT$_7$ receptor seems to be expressed in a highly discrete manner in several brain areas and in the digestive tract, as shown by Northern blots and *in situ* hybridization analysis of gene transcripts. It is not entirely clear, however, whether the receptor protein was previously detected in radioligand or functional studies. Being easily labeled by [^{3}H]5-HT, the 5-HT$_7$ receptor might have been considered as a member of the heterogeneous "5-HT$_{1nonA,\ nonB,\ nonC}$" subfamily of recognition sites (23). In fact, its pharmacology seems to significantly differ from that of any of the various members that were tentatively identified in [^{3}H]5-HT binding experiments, performed in the presence of masking drugs.

Also the possibility that the 5-HT$_{1A}$ receptor may be coupled to adenylyl cyclase, not only negatively but also positively, was evoked in view of the observations of multiphasic activation of the enzyme by 5-HT in membranes from various rodent brain areas (24, 25, 46), among which is the hippocampus that highly expresses the 5-HT$_7$ receptor

(Figs. 6 and 7). It is clear, however, that the amino acid sequence and pharmacology of the 5-HT_7 receptor differ from those of the cloned 5-HT_{1A} receptor (6).

It seems also to differ from that of the cerebral receptor whose stimulation by 5-HT in nanomolar concentrations stimulates the cyclase (26). The pharmacology of 5-HT_7 does not correspond to that of the 5-HT_4 receptor positively coupled to adenylyl cyclase, namely by its low affinity for ICS 205930 or benzamides such as metoclopramide (19) and that of the 5-HT receptor responsible for glycogenolysis (47).

In summary, it seems that the native 5-HT_7 receptor has never been detected in cerebral membranes but this is not surprising in view of the multiplicity of [^{3}H]5-HT binding sites as well as of 5-HT receptors coupled to the cyclase and its presumably low abundance as judged from the discrete expression of the 5-HT_7 gene transcripts (Figs. 6 and 7).

The 5-HT_7 receptor seems to be mainly associated with limbic brain divisions receiving serotoninergic inputs (e.g., the hippocampus, amygdaloid complex, or mammillary nuclei). This suggests a role for the 5-HT_7 receptor in the control of serotoninergic functions associated with these areas (e.g., learning, mood, neuroendocrine, or vegetative behaviors). Consequently, selective ligands for the receptor, when designed, might be useful in pathological conditions such as depression, anxiety, or obesity that may involve the same cerebral areas.

Interestingly, some neuronal populations (e.g., the pyramidal cells in CA_2 or CA_3 of Ammon's horn) seem to express both 5-HT_7 and 5-HT_{1A} receptors (48), which mediate opposite cAMP responses. From a functional point of view, it will be important to determine whether the same neurons or distinct subpopulations express the two subtypes.

It will also be of interest to establish whether 5-HT_7 receptor mRNAs detected in raphe nuclei reflect the local synthesis of another class of 5-HT autoreceptors.

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